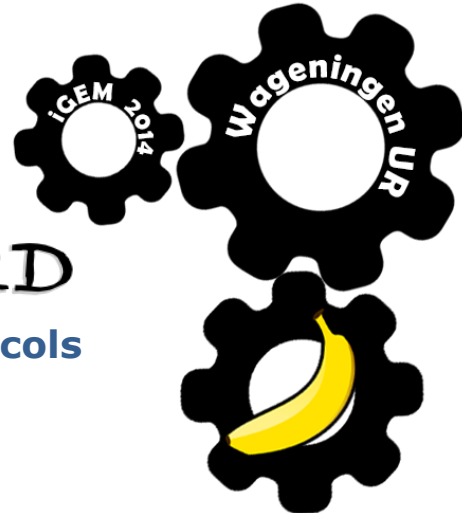




GUARD

Protocols



Restriction and Ligation

Restriction Digestion

(Adjusted protocol based on New England Biolabs NEB Biobrick Assembly Kit protocol)

COMPONENT	20 µl REACTION
Plasmid or DNA fragment to digest	1 µg
Restriction Enzyme I	1 µl
Restriction Enzyme II	1 µl
10X NEBuffer 2.1*	5 µl
Water, nuclease-free	to 20 µl

* double digestions specific buffer has to be checked on line in the New England Biolabs website

- Incubate restriction digestion at 37°C for at least 1 hour.
- Deactivate the enzymes at 80°C for 20 minutes.

Ligation

(Adjusted protocol based on New England Biolabs Biobrick Assembly Kit protocol)

- Set up the following reaction in a microcentrifuge tube on ice.
- Gently mix the reaction by pipetting up and down and microfuge briefly.
- For cohesive (sticky) ends, incubate at least 20 minutes, optimally overnight at room temperature.

COMPONENT	20 µl REACTION
10X T4 DNA Ligase Buffer*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	62 ng (0.060 pmol)
Water, nuclease-free	to 19 µl
T4 DNA Ligase	1 µl (dilute 5 times)

T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes. Use NEBioCalculator to calculate molar ratios.

